BeadRetriever™



BeadRetriever™

User Manual

User Manual Rev. 05; November 12, Product No. 159.50

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CONTENTS

TABLE OF CONTENTS

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The BeadRetriever has national and international patents as well as patents pending. The production of Dynabeads is covered by various patents.

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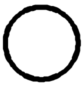
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1 SAFETY SYMBOLS AND MARKINGS

These symbols are intended to draw your attention to particularly important information and alert you to the presence of hazards as indicated.

SAFETY SYMBOLS USED IN THE BEADRETRIEVER

Power ON



Power OFF

SN

Serial number

REF

Catalog number



Date of manufacture

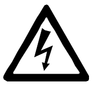


Consult Instructions for Use



WEEE symbol This product is required to comply with the European Union’s Waste Electrical & Electronic Equipment (WEEE) Directive 2012/19/EC.

WARNING MARKINGS USED IN THE DOCUMENTATION



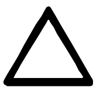
Caution: risk of electric shock.



Caution: biohazard risk.



Caution: risk of personal injury to the operator or a safety hazard to the surrounding area.



Caution: risk of damage to the instrument, other equipment or loss of performance or function in a specific application.

2 ABOUT THE USER MANUAL

This User Manual has been written for the actual user (e.g., laboratory technician) and provides information on the BeadRetriever magnetic bead processor, including the installation and operating instructions.

Read the manual in its entirety prior to operating the instrument.

This User Manual has been designed to give you the information you need to:

- Install the BeadRetriever
- Use the BeadRetriever in routine jobs – the processing step
- Perform basic maintenance procedures.

This User Manual also describes all the features and specifications of the BeadRetriever hardware and on-board software.

APPENDIX A. Application Protocols for BeadRetriever using Dynabeads® for selective concentration of target microorganisms explains the processing principles and procedures.

In Chapter 9 TROUBLESHOOTING GUIDE you will find explanations of all error messages and a problem-solving guide. The user should be familiar with the contents of Chapter 7 on maintenance.

For warranty and ordering information, refer to Chapter 10 WARRANTY and Chapter 11 ORDERING INFORMATION.

A CD-ROM video demonstrating use of the BeadRetriever and automated IMS (AIMS) is available to all users. Please request a CD by email: environmental@invitrogen.com or visit www.invitrogen.com/microbiology.

3 INTRODUCTION TO THE BEADRETRIEVER

3.1 Intended use

The BeadRetriever magnetic bead processor (*Fig. 3.1*) is intended for professional research and laboratory use by trained personnel. The instrument is intended for automated transfer and processing of magnetic beads in a 1 ml tube scale. Use for self-testing is excluded.

3.2 Principle of operation

The principle of the BeadRetriever system (patents pending) is based on the use of magnetic rods covered with disposable, specially designed tip combs and tube strips. The instrument functions without any dispensing or aspiration parts or devices.

Samples and reagents including magnetic beads are dispensed into the tube strips according to the corresponding kit instructions. The steps of the protocol that are selected by the user via the keypad and display have already been preloaded in the on-board software.



Fig. 3.1 BeadRetriever magnetic bead processor

3.3 Advantages of processing magnetic beads with BeadRetriever

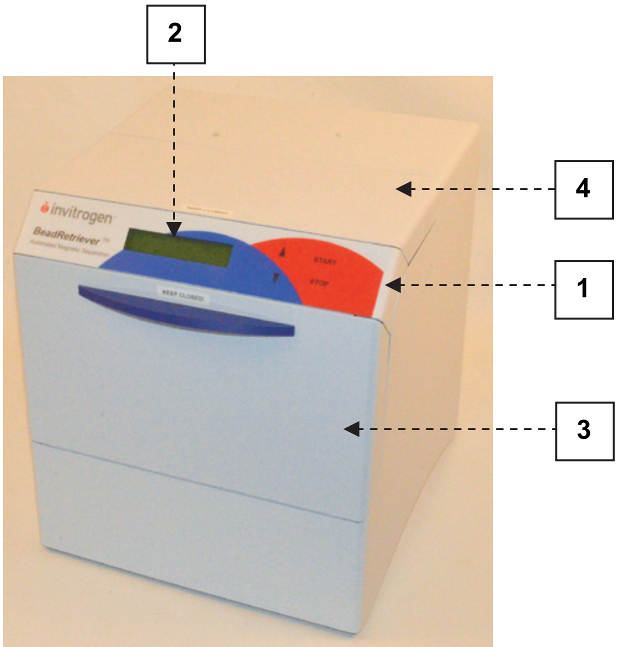
The BeadRetriever magnetic bead processor provides several advantages relating mainly to the principle of operation in that it:

- Enables automation of complicated manual steps
- Enables simultaneous processing and purification
- Enables a quicker reaction and a more efficient wash due to the simultaneous shaking during incubation and washing
- Facilitates a good collection of bead-bound sample due to the efficiency of the magnet
- By moving the beads from tube to tube during processing, contaminating background flora is reduced further
- Prevents carryover due to the fact that the same tip is only used for one sample
- Facilitates the whole processing with the aid of an internal program.

4 FUNCTIONAL DESCRIPTION

4.1 Instrument layout

4.1.1 Front view



1 Keypad
2 Display
3 Front lid
4 Top lid

Fig. 4.1 BeadRetriever front view with lids closed

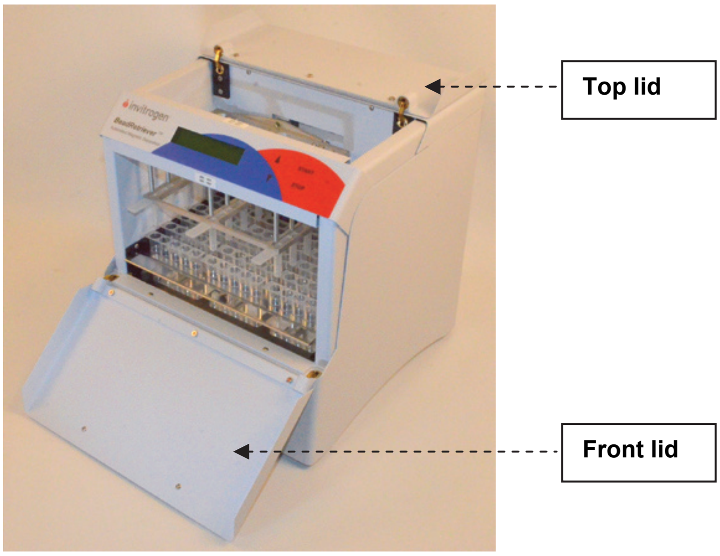
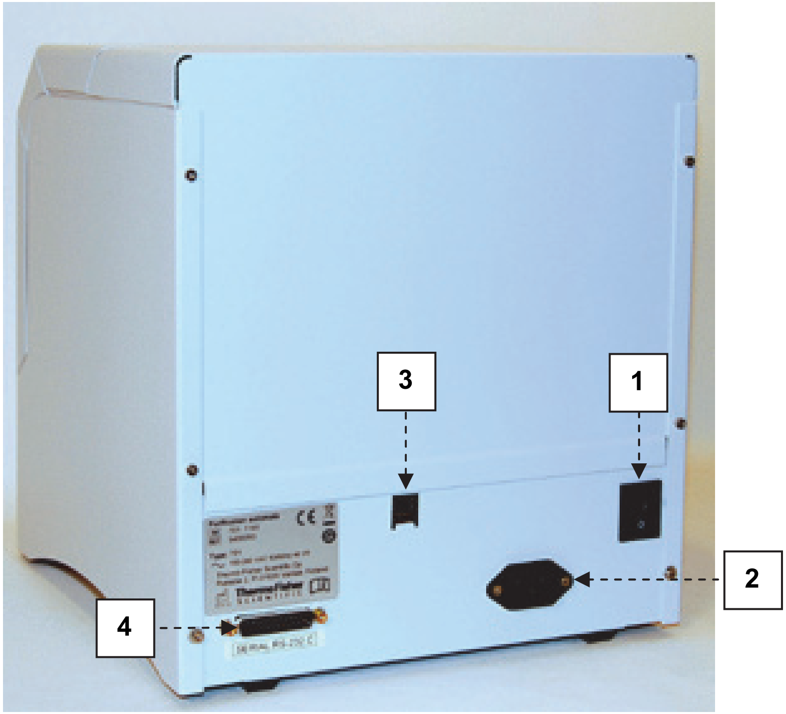


Fig. 4.2 BeadRetriever front view with lids open

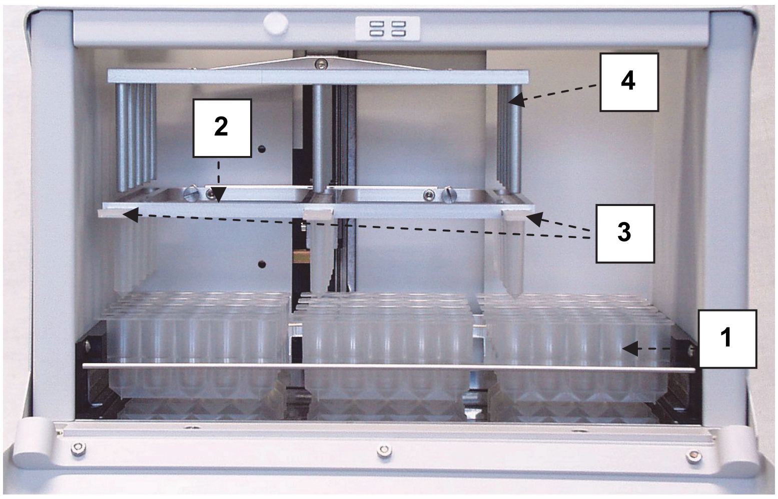
4.1.2 Back view



1 ON/OFF switch
2 Main power supply socket
3 USB
4 Serial port RS-232C

Fig. 4.3 BeadRetriever rear view

4.1.3 Internal view



1 Tube-strip tray with tube strips
2 Tip comb holder
3 Tip comb slot with tip comb
4 Magnetic rod

Fig. 4.4 BeadRetriever internal view

4.2 BeadRetriever magnetic bead processor

The BeadRetriever instrument (*Fig. 4.5*) is designed for maximum 15 tube strips, which are compatible with the tip comb. The tube strip(s) is (are) kept stationary and the only moving assembly is the processing head with tip combs and magnetic rods. The head consists of two vertically moving platforms. One is needed for the magnetic rods (3 x 5 pieces) and the other one for the plastic tip combs (*Fig. 4.4*).

One tube-strip tray contains fifteen separate tube strips and one sample uses one tube strip which is made up of five tubes. One tip comb containing five tips is used for processing five samples at a time.

Before starting the magnetic bead processing via the keypad and display, the samples and reagents are dispensed into the tubes within the tube strips and the tip comb(s) is (are) loaded into its (their) slot(s). The tube strip(s) is (are) placed into the removable tray in the correct position and the tray is pushed into the end position. During the operation, the front and top lids can be closed (*Fig. 4.1*) or open (*Fig. 4.2*). Closed lids protect the processing against environmental contamination.

The operating principle employed is inverse magnetic bead processing technology (*Fig. 4.6*). Rather than moving the liquids, the magnetic beads are moved from tube to tube containing specific reagents, in contrast to the external magnet method. Magnetic beads are transferred with the aid of magnetic rods covered with disposable, specially designed plastic tip combs.

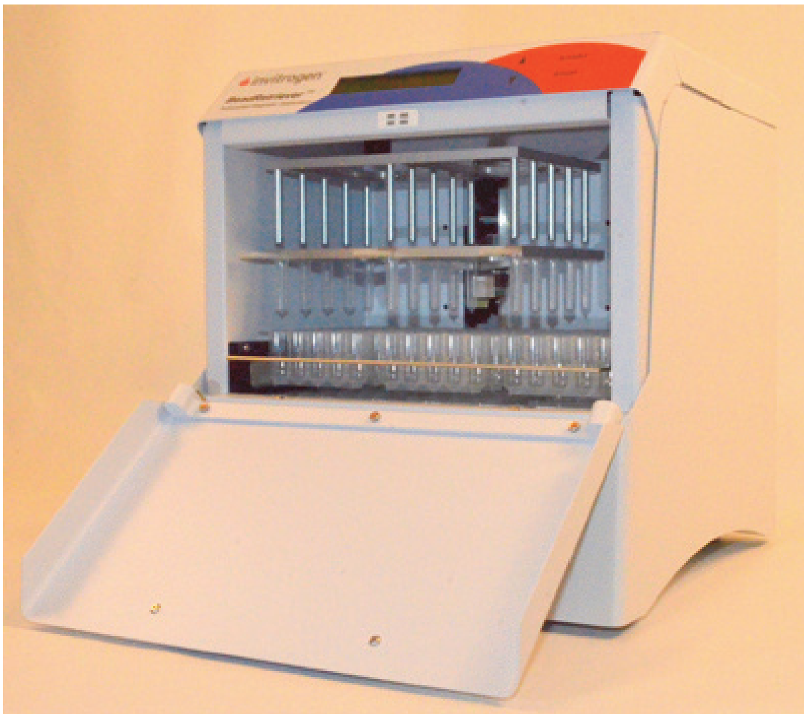
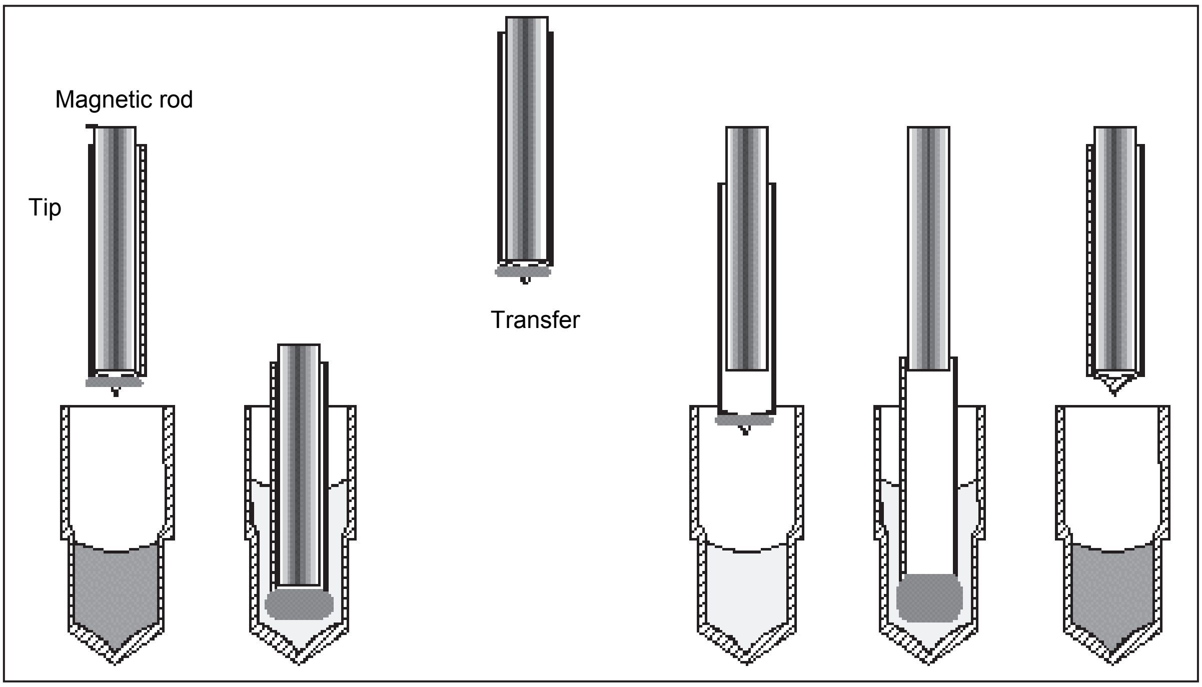


Fig. 4.5 BeadRetriever magnetic bead processor

4.3 Principle of magnetic bead processing



Magnetic bead suspension before collection | Collection of magnetic beads | Magnetic beads on the tip surface | Release of magnetic beads | Ready suspension

Fig. 4.6 Inverse magnetic bead processing

4.4 Working with a magnetic rod

Working with magnetic beads can be divided into five separate processes:

- collecting beads
- releasing beads
- washing beads
- incubation
- concentration

4.4.1 Collecting magnetic beads

During the collection of the magnetic beads, the magnetic rod is fully inside the tip. The magnetic rods together with the tip comb(s) move slowly up and down in the tubes within the tube strip and the magnetic beads are collected onto the wall of the tips. The magnetic rod together with the tip comb, having collected the magnetic beads, will lift out of the tube and be transferred into the next tube within the same tube strip.

4.4.2 Releasing magnetic beads

After collection of the magnetic beads, the magnetic rods together with the tip comb(s) are lifted from the tubes, the magnetic rods are lifted up and the tip comb(s) is (are) lowered into the next tube in the tube strip containing a reagent.

Magnetic beads are released by moving the tip comb(s) up and down several times at considerably high speed until all the beads have been mixed with the substance in the next reaction.

4.4.3 Washing magnetic beads

Washing the magnetic beads is a frequent and an important processing phase. Washing is a combination of the release and collection processes in a tube filled with washing solution.

To maximize washing efficiency, the magnetic rods together with the tip comb(s) are designed to have minimized liquid-carrying properties.

4.4.4 Incubation

To keep the magnetic bead suspension evenly mixed in long-running reactions, the tip comb(s) can be moved up and down from time to time.

4.4.5 Changing the volume during the magnetic bead processing

The volume of the first tube can be larger than the volume of the next tube and this is used for *concentration* purposes (see Fig. 4.7 below).

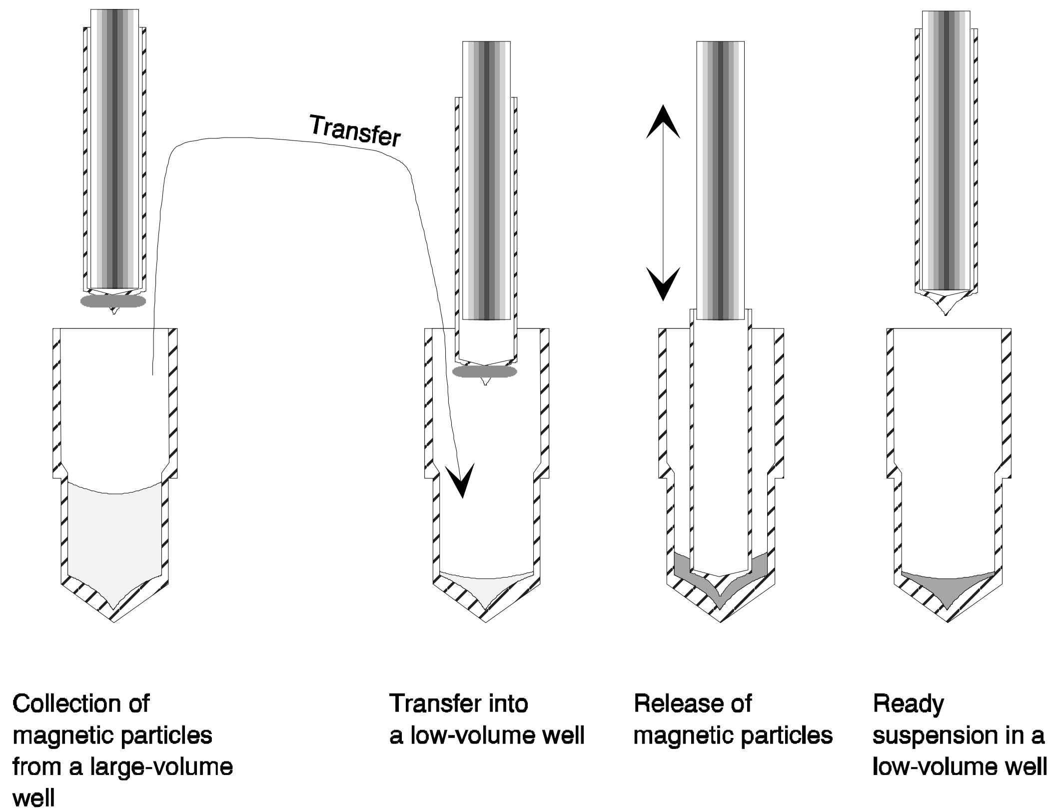


Fig. 4.7 A concentration step during magnetic bead processing

5 INSTALLATION

5.1 What to do upon delivery

5.1.1 How to unpack

Move the unpacked instrument to its site of operation. Unpack the BeadRetriever instrument and accessories carefully with the arrows on the transport package pointing upwards. The following notes and instructions are sent with the instrument and are immediately available when you open the package:

- the packing instructions/packing list
- the BeadRetriever Feedback Form
- the Warranty Certificate card
- the User Manual.

Caution: DO NOT touch or loosen any screws or parts other than those specially designated in the instructions. Doing so might cause misalignment and will void the instrument warranty.

To lift the instrument, put your fingers under the bottom on either sides and lift it with your back straight.

Retain the original packaging for future transportation. The packaging is designed to assure safe transport and minimize transit damage. Use of alternative packaging materials may invalidate the warranty. Also retain all instrument-related documentation provided by the manufacturer for future use.

5.1.2 Checking delivery for completeness

Check the enclosed packing list against order. If any parts are missing, contact Invitrogen or your local Invitrogen representative.

5.1.3 Checking for damage during transport

Visually inspect the transport package, the instrument and the accessories for any possible transport damage.

If the carton has been damaged in transit, it is particularly important that you retain it for inspection by the carrier in case there has also been damage to the instrument.

Visually check all interconnections in the basic instrument. Check that there are no loose parts inside the instrument.

If any parts are damaged, contact Invitrogen or your local Invitrogen representative.

5.1.4 Environmental requirements

When you set up your BeadRetriever, avoid sites of operation with excess dust, vibrations, strong magnetic fields, direct sunlight, draft, excessive moisture or large temperature fluctuations.

- Make sure the working area is flat, dry, clean and vibration-proof and leave additional room for cables, lids, etc.
- Make sure that there is sufficiently room behind the instrument to enable disconnecting the device.
- Make sure the ambient air is clean and free of corrosive vapors, smoke and dust.
- Make sure the ambient temperature range is between +10°C (50°F) and +40°C (104°F).
- Make sure relative humidity is between 10% and 90% (non-condensing).

Leave sufficient space (at least 10 cm) at both sides and at the back of the unit to allow adequate air circulation.

The BeadRetriever does not produce operating noise at a level that would be harmful. No sound level measurements are required after installation.

Warning:
DO NOT operate the instrument in an environment where potentially damaging liquids or gases are present

5.1.5 Things to avoid

DO NOT smoke, eat or drink while using the BeadRetriever. Wash your hands thoroughly after handling test fluids. Observe normal laboratory procedures for handling potentially dangerous samples. Use proper protective clothing. Use disposable gloves. Be sure the working area is well ventilated.

Never spill fluids in or on the equipment.

Warning: This product contains strong permanent magnets.
People wearing a pacemaker or metallic prostheses should not use this product.
A pacemaker or prostheses may be affected or damaged if it comes in very close contact with a strong magnetic field.

5.1.6 Technical prerequisites

Place the instrument on a normal laboratory bench. The net weight of the unit is approx. 10 kg (23 lbs.).

The instrument operates at voltages of 100–240 V a.c. The frequency range is 50/60 Hz.

5.2 Setups before you put the instrument into operation

5.2.1 How to release the transport locks

Tools: 3 mm flat-head screwdriver (supplied with the instrument)

Note: THERE ARE FOUR (4) TRANSPORT LOCKS (SCREWS) PRESENT (*Fig. 5.2*). A white tag with the red text ‘**CAUTION! Remove Transport Lock Before Use**’ is fastened to the instrument to

attract the user's attention) (*Fig. 5.1*). The tag in *Fig. 5.1* and the black arrows in *Fig. 5.2* indicate where the transport locks are situated. Remove the tag and release the transport locks. Ensure that the transport locks have been released before you put the instrument into operation.

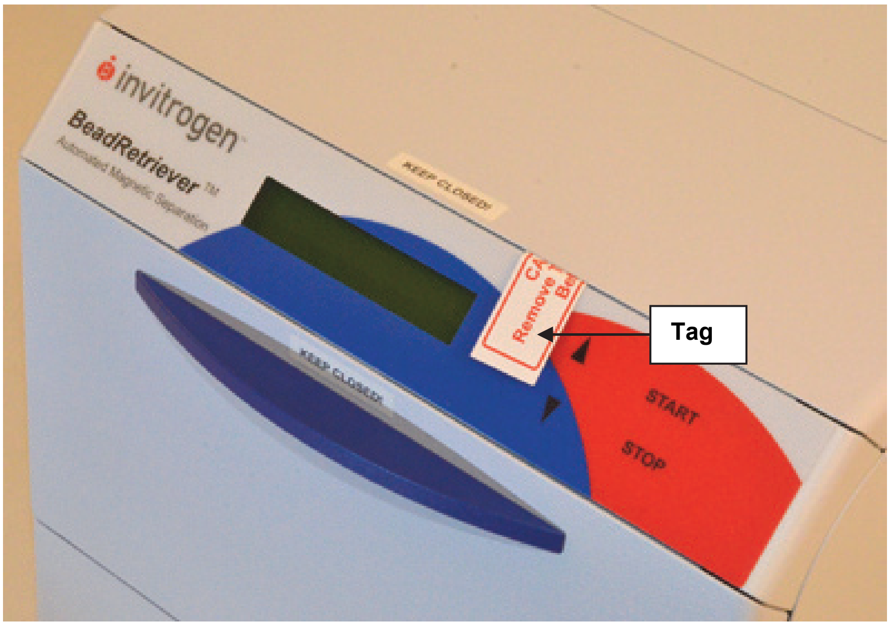


Fig. 5.1 BeadRetriever transport lock tag present before release of the locks

1. To remove the transport locks, unscrew the four (4) screws (*Fig. 5.2*) with the 3 mm flat-head screwdriver supplied. Make sure that the screws are fully unscrewed in the opposite direction.

2. Then remove the transport lock warning tag (*Fig. 5.1*).

3. Save the transport lock warning tag for future transportation.

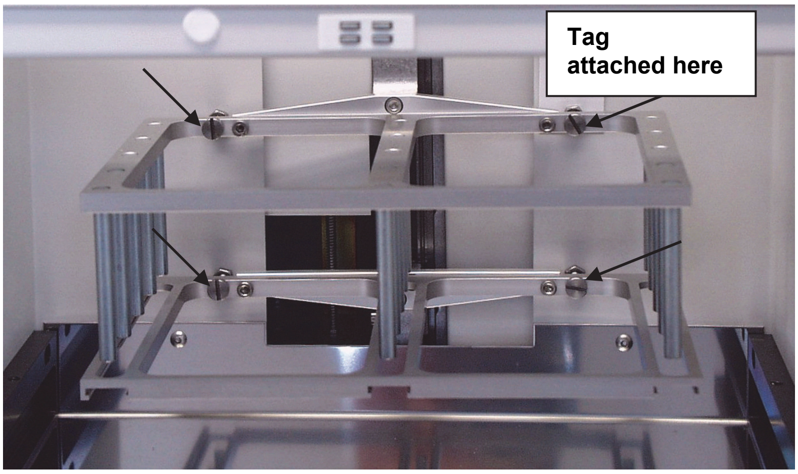


Fig. 5.2 Location of the transport lock screws and warning tag

5.2.2 How to ensure startup

1. **Warning:** Ensure that the mains switch (*Fig. 4.3*) on the bottom left of the back panel is in the OFF position.

2. Connect the mains supply cable to the *mains power socket* (*Fig. 5.3*) at the bottom left of the back panel. If you need to use any other type of mains supply cable than supplied, use only cables certified by the local authorities. Before you plug in the power cable, ensure that the voltage on the rating label at the bottom left of the back panel (*Fig. 4.3*) corresponds to the local voltage.

3. Connect the instrument to a correctly installed line power outlet that has a protective conductor that is grounded.

Warning: Never operate your instrument from a power outlet that has no ground connection.



Fig. 5.3 Connecting the mains supply cable

5.3 Operational check

1. First switch the instrument ON. The instrument performs initialization tests and adjustments. The initialization tests are so-called self-tests.

2. The display quickly shows the internal software version and after that, the display shows one of the protocols in the internal memory. This happens when the initialization tests and adjustments have been completed.

3. It is recommended that you carry out an empty run (e.g., a demonstration) to verify proper instrument operation.

6 ROUTINE OPERATION

6.1 Switching on

Before you switch on the BeadRetriever, ensure that the voltage on the rating label at the bottom left of the back panel (*Fig. 4.3*) corresponds to the local voltage.

6.2 How to use the keypad and display

The liquid crystal display (LCD) is a 20 character by 2 line LCD.

The lower line of the display can show the steps of the protocol, while the processing is running. The remaining time till the end of the process is shown in the top right corner. Furthermore, if errors occur, they will be displayed on the lower line.

The keypad and display are shown in *Fig. 6.1*.



Fig. 6.1 BeadRetriever keypad

The keys available are:

- *two cursor keys* (or *arrow keys*, *direction keys*): to select the processing step to be run

▲	To change to the previous/next protocol by scrolling up.
▼	To change to the next protocol by scrolling down.

- *two function keys*

START	To initiate the processing step.
STOP	To pause/terminate the processing step. In short: STOP (paused)/START (the instrument continues after a pause step) STOP (paused)/STOP (the processing is terminated).

6.3 How to START

- First of all slide the tube-strip tray out (*Fig. 6.2*).

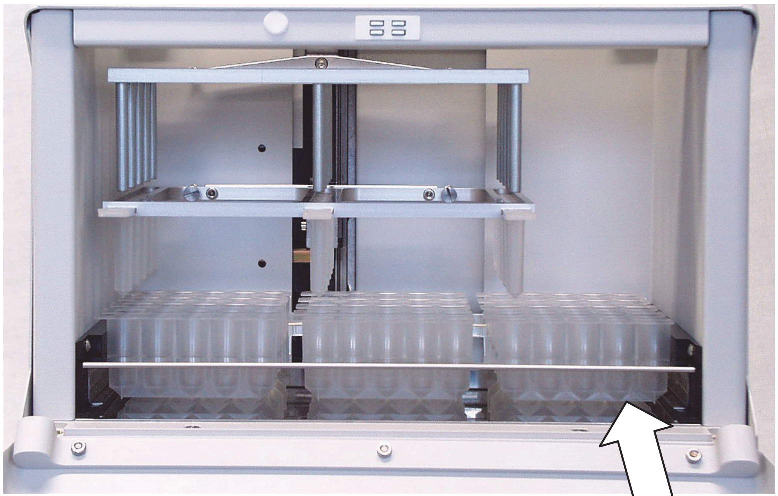


Fig. 6.2 Loading the tube-strip tray

- A tube strip contains one sample and the reagents for a single processing. The user can select the position of the tube strip(s) on the tube-strip tray. The user must keep track of the sample order. However, the orientation of the tube strip is fixed. The tube strips have to be positioned so that the slip ends face left (*Fig. 6.3* and *Fig. 6.5*). Note that the order in the tube strip of the sample and reagents is determined by the kit used. Load only maximum fifteen special tube strips at a time into the tray for processing (*Fig. 6.5*). Slide the tray fully back in.

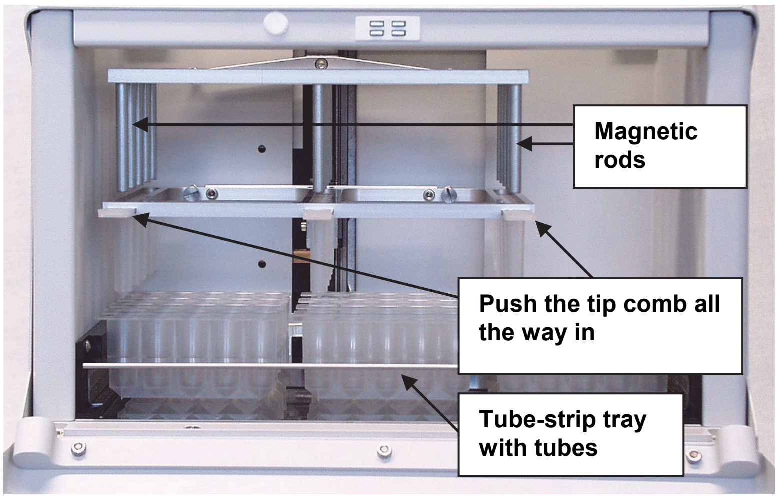


Fig. 6.3 Positioning of the tube-strip tray and insertion of the tip comb(s)

- The tip comb(s) always has (have) to be in its (their) slot(s) and fully inserted (*Fig. 6.3*). The instrument also functions with either one tube strip or one to fifteen tube strips depending on the amount of samples. At least one tip comb (*Fig. 6.4*) always has to be inserted into one of the narrow slots meant for the tip comb.

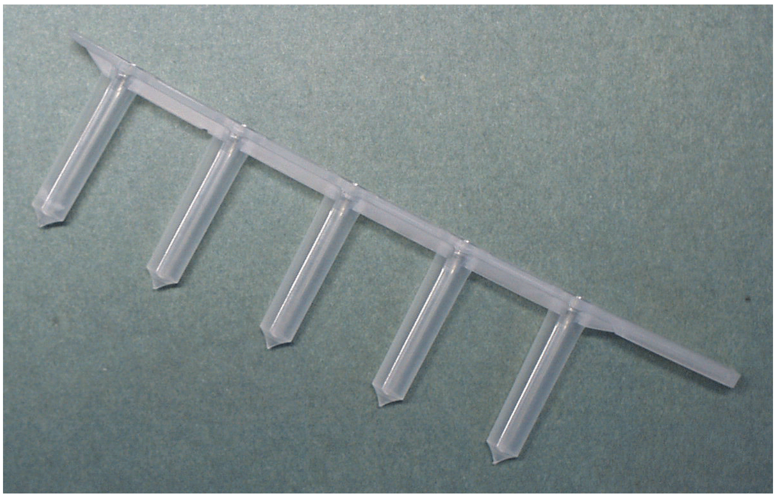


Fig. 6.4 Tip comb specially designed for the BeadRetriever

- Close the front lid if you want to. The front lid protects the instrument against environmental contamination.

Note: The front lid and/or top lid can be left open if desired. This action does not break the run.

- Select the name of the method.

- Use the **START** key to initiate the run.

For startup refer to Section 6.1 Switching on.

Note: At the end of the run, make sure that the text "*End of run*" is shown on the display, and then press the **STOP** key.

Note: The samples are collected immediately after the run and transferred into other tubes for longer storage.

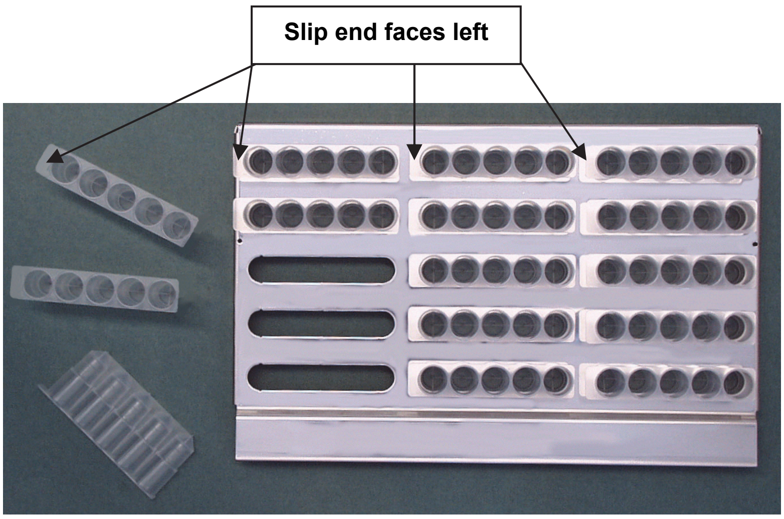


Fig. 6.5 Tube-strip tray containing tubes specially designed for the BeadRetriever

6.4 Shutdown

Switch the BeadRetriever off by pressing the power switch (*Fig. 4.3*) at the bottom left of the back panel of the instrument into the OFF position.



Remove any tube strips still on the tube-strip tray. Dispose of all tube strips as biohazardous waste.



Remove any tip combs still in the instrument. Dispose of tip combs as biohazardous waste.

Wipe the tray surface and the adjacent instrument surface with a soft cloth or tissue paper moistened with distilled water, a mild detergent (SDS, sodium dodecyl sulfate) or soap solution.

If you have spilt infectious agents on the tray, disinfect with 70% alcohol or some other disinfectant (see Section 7.3 Decontamination procedure).

7 MAINTENANCE

7.1 Regular and preventive maintenance

For reliable daily operation keep the instrument free of dust and liquid spills.
Abrasive cleaning agents are not recommended, because they are likely to damage the paint finish.

It is recommended that you clean the case of the instrument periodically to maintain its good appearance. A soft cloth dampened in a warm, mild detergent solution will be sufficient.

Clean the outside of the instrument and the tube-strip tray with clean low-pressure compressed air or a cloth dampened with water or a mild detergent when necessary.

Although the BeadRetriever is constructed from high-quality materials, you must immediately wipe away spilt saline solutions, solvents, acids or alkaline solutions from outer surfaces to prevent damage.

Painted surfaces can be cleaned with most laboratory detergents. Dilute the cleaning agent as recommended by the manufacturer. DO NOT expose painted surfaces to concentrated acids or alcohols for prolonged periods of time as damage may occur.

Clean the display areas with a mild laboratory detergent. The keypad has a wipe-clean surface.

Plastic covers and surfaces can be cleaned with a mild laboratory detergent or alcohol.

If any surfaces have been contaminated with biohazardous material, a mild sterilizing solution should be used.

DO NOT autoclave any part of this instrument except for the tube-strip tray.

7.1.1 How to clean the tube-strip tray

Keep the tube-strip tray surface clean to avoid dust and dirt entering into the instrument. Clean the tray surface at least once a week using a soft cloth or tissue paper soaked in a mild detergent solution (SDS), soap solution or alcohol.

If you have spilt infectious agents on the tube-strip tray, you can autoclave the tray.

7.1.2 How to clean the magnetic rods

If required, wipe the magnetic rods using a soft cloth or tissue paper soaked in a mild detergent solution (SDS), soap solution or alcohol.

7.1.3 How to clean the processing chamber

If required, clean the processing chamber using a soft cloth or tissue paper soaked in a mild detergent solution (SDS), soap solution or alcohol.

7.2 Disposal of materials

Refer to local regulations for the disposal of infectious material.

The samples can be potentially infectious. Dispose of all used tube strips, tip combs, disposable gloves, syringes, disposable tips, etc. as biohazardous waste.

7.3 Decontamination procedure

If there is any risk of contamination with biohazardous material, the procedure recommended below or some other corresponding decontamination procedure must be performed.

It is strongly recommended that the complete decontamination procedure be performed before relocating the instrument from one laboratory to another.

Decontamination is not required for the proper functioning of the instrument.

Example of decontaminants

- Ethanol 70%
- Virkon® solution 1–3%
- Glutaraldehyde solution 4%
- Chloramine T
- Microcide SQ® 1:64
- Decon® 90 min. 4%

Always use disposable gloves and protective clothing and operate in a well-ventilated area.

1. Prepare the decontaminating agent: for example, 200 ml 4% glutaraldehyde solution (or another agent recommended by your safety officer).

2. Empty the tube-strip tray.

3. Switch OFF the power and disconnect the mains supply cable (*Fig. 5.3*).

4. Disinfect the outside of the instrument using a cloth dampened with 70% ethanol.

5. Place the instrument in a large plastic bag. Ensure that the lids are open.

6. Place a cloth soaked in the prepared solution into the bag. Ensure that the cloth does not make contact with the instrument.

7. Close the bag firmly and leave the instrument in the bag for at least 24 h.

8. Remove the instrument from the bag.
9. Clean the instrument using a mild detergent.
10. Remove any stains using 70% ethanol.
11. After performing this decontamination procedure, label the instrument with a signed and dated Certificate of Decontamination.

7.4 How to pack for service

When you ship the instruments for service remember to:

- Inform about the use of hazardous materials.
- Decontaminate the instrument beforehand.
- Install the transport locks.
- Pack the instrument according to the enclosed packing instructions.
- Use the original packaging to ensure that no damage will occur to the instrument during shipping. Any damage will incur additional labor charges.
- Enclose a dated and signed Certificate of Decontamination (see Section 9.4.1.1) both inside and attached to the outside of the package, in which you return your instrument (or other items).
- Enclose the return authorization number (RGA) given by the Invitrogen representative.
- Indicate the fault after you have been in touch with your local Invitrogen representative
- See Section 8.1 for details on storage and transportation temperatures.

7.5 Service contracts

It is recommended that you maintain and service the instrument regularly every twelve (12) months on a contract basis by the manufacturer's trained service engineers. This will ensure that the product is properly maintained and gives trouble-free service. Contact your local Invitrogen representative for details.

7.6 Disposal of the instrument



- Decontaminate the instrument prior to disposal. See Sections 7.3, 9.4.1 and 9.4.1.1 on decontamination.
- Dispose of the instrument according to the legislation stipulated by the local authorities concerning take-back of electronic equipment and waste. The proposals for the procedures vary by country.
- Regarding the original packaging and packing materials, use the recycling operators known to you.
- For more information, contact your local Invitrogen office or local supplier.

8 TECHNICAL SPECIFICATIONS

8.1 General specifications

Technical specifications

Overall dimensions	*ca.* 290 mm (W) x 290 mm (D) x 310 mm (H) [11.4 in. (W) x 11.4 in. (D) x 12.2 in. (H)]
Weight	*ca.* 10 kg (23 lbs.)
Operating conditions (Indoor use)	+10°C – +40°C, RH: 90% max.
Transportation conditions	-40°C – +70°C, packed in transport packaging
Storage conditions	-25°C – +50°C, packed in transport packaging
Mains power supply	100–240 V a.c., 50/60 Hz, nominal Automatic voltage detection
Power consumption	40 VA max.
Heat dissipation	136 BTU max.
Computer interface	Serial RS-232C port. USB.

Performance specifications

Tube-strip tray (autoclavable)	Special design, 3 x 5 format
Magnetic rods (fixed)	3 x 5 format
Capacity	15 samples/run (3 x 5)
Vessel volumes	50 µl – 1 ml
Vessel type (disposable)	Special tube strip, 1 x 5 tubes
Tip comb (disposable)	Special design, 1 x 5 format
Magnetic bead size	*ca.* > 1 µm
Collecting efficiency	95%/vessel (washing buffer)
Incubating temperature	Ambient temperature (i.e., no incubation)
Keyboard / Display	START/STOP/two cursor keys/LCD
Software	Space for *ca.* 200 protocols (loading through the external PC)

8.2 Safety specifications

This section describes the safety specifications for the BeadRetriever instrument.

8.3 In conformity with the requirements

BeadRetriever bears the following markings:

Type 701
100–240 V a.c. 50/60 Hz, 40 VA
CE mark

BeadRetriever conforms to the following requirements:

2006/95/EC (Low Voltage Directive)
2004/108/EC (Electromagnetic Compatibility Directive, EMC)
FCC Part 15, Subpart B/Class B (July 2004)
2012/19/EC (Waste of Electrical and Electronic Equipment)
2011/651/EC (RoHS Directive – Restriction of the Use of Certain Hazardous Substances in Electrical and Electronic Equipment)
2006/42/EC (Machinery Directive)

The safety specifications are also met under the following environmental conditions in addition to or in excess of those stated in the operating conditions:

Altitude:	Up to 2000 m
Temperature:	+5°C to +40°C
Humidity:	Maximum relative humidity 80% for temperatures up to 31°C decreasing linearly to 50% relative humidity at 40°C
Mains supply fluctuations:	± 10% from nominal
Installation category (overvoltage category):	II according to IEC 60664-1 (see **Note** 1)
Pollution degree:	2 according to IEC 60664-1 (see **Note** 2)

Notes

1. The *installation category* (overvoltage category) defines the level of transient overvoltage, which the instrument is designed to withstand safely. It depends on the nature of the electricity supply and its overvoltage protection means. For example, in CAT II, which is the category used for instruments in installations supplied from a supply comparable to public mains, such as hospital and research laboratories and most industrial laboratories, the expected transient overvoltage is 2500 V for a 230 V supply and 1500 V for a 120 V supply.

2. The *pollution degree* describes the amount of conductive pollution present in the operating environment. Pollution degree 2 assumes that normally only nonconductive pollution, such as dust, occurs with the exception of occasional conductivity caused by condensation.

9 TROUBLESHOOTING GUIDE

9.1 Error messages and warnings

Error message	*Warning*
Command error Cannot move track Head position error Head timeout Track position error Track timeout Magnets pos. error Magnets timeout Serial number error Serial no already set Too high pos. offset Invalid parameter Out of memory No acceleration tbl NV parameters lost Program full	Time already expired

9.2 Troubleshooting guide

Error	***Cause***	***Action***
• Cannot move track	The track cannot be moved because the head is not up.	Press STOP. If the fault cannot be remedied, then switch the power on and off.
• Head position error	The calculated head position does not agree with the head home sensor signal.	Check that there is nothing preventing the head from moving up and down.
• Head timeout	The head has not arrived at the home sensor within the allowed time.	Check that there is nothing preventing the head from moving up and down.
• Track position error	The calculated track position does not agree with the track home sensor signal.	Check that there is nothing preventing the track from moving left or right.
• Track timeout	The track has not arrived at the home sensor within the allowed time.	Check that there is nothing preventing the track from moving left or right.
• Magnets position error	The calculated magnetic rod position does not agree with the rod home sensor signal.	Check that there is nothing preventing the magnetic rods from moving up and down. Ensure that the tip comb is fully in.
• Magnets timeout	The magnetic rods have not arrived at the home sensor within the allowed time.	Check that there is nothing preventing the magnetic rods from moving up and down.
• Nonvolatile parameters lost	The nonvolatile parameters of the instrument are lost.	Contact authorized service.

9.3 Frequently asked questions (FAQ) about BeadRetriever processing

Q1: **What does the BeadRetriever do?**

A1: Dynabeads-based products for microbiology have been designed for automated immunomagnetic separation (AIMS) of these target organisms from food, feed and environmental samples. The process can selectively concentrate the specific pathogenic microorganisms from sample materials. Invitrogen now offers a complete BeadRetriever system (the magnetic bead processor and the reagents) for the purification of microbial samples. The processor handles beads automatically according to the preloaded protocol.

Q2: **Do I need a PC to control the processor?**

A2: The protocols are preloaded by Invitrogen and no PC is needed for routine use. A PC program can be used to create new protocols or modify existing protocols if needed. PC-modified protocols are then downloaded into the processor for routine use.

Q3: **Will the magnets get weaker? If so, how long can they be used? Can they be replaced?**

A3: The BeadRetriever magnets are made of material that is very stable. The magnetic field will not get weaker.

Magnets can be replaced if needed. Usually there is no need to replace them, but it can be done, e.g., if they are mechanically damaged during runs. Local technical personnel can carry out changing the magnets.

Q4: **How strong are the magnets? Can they disturb, e.g., some sensitive equipment?**

A4: The BeadRetriever should not be kept in close proximity to magnetic tapes, computer discs or other magnetic storage systems, such as credit cards, as these can be damaged by the strong magnetic field of the BeadRetriever heads.

Do not hold the BeadRetriever heads close to a PC display, since this may cause damage to the display. Do not use metal tools when handling BeadRetriever heads.

Q5: **What happens if I forget to insert the tip combs into the tip comb holder and the magnetic beads stick to the magnetic rods?**

A5: Wipe the magnetic rods using a soft cloth or tissue paper soaked in a mild detergent solution, soap solution or alcohol.

Q6: **What if the magnetic beads remained in the well?**

A6: If the starting material is too viscose, the magnetic rods will not be able to collect the beads. Dilute the sample and check that the sample is properly homogenized/lysed.

Q7: **What if magnetic beads are attached to the tip combs?**

A7: This happens sometimes but it will not affect the yield because the sample has been released from the beads.

Q8: **Are the volumes of reagents in each well critical?**

A8: It is strongly recommended that you keep the given volumes within certain limits to avoid failure in performance of the chemical reactions and the processor.

9.4 Service request protocol

If the BeadRetriever requires service, contact your local Invitrogen representative. DO NOT under any circumstances send the instrument for service without any prior contact. It is imperative to indicate the fault and nature of the required service. This will ensure a faster return of the instrument to the customer.

The Invitrogen representative will prepare a complaint form (Complaint-order) containing a detailed description of the fault, symptom or condition based on the information you give them.

Check Section 7.4 How to pack for service. You will find instructions on how to proceed before shipping the instrument for service.

Check that any necessary decontamination procedure has been carried out before packing. See Sections 9.4.1 and 9.4.1.1 on decontamination. Refer also to Section 7.3 Decontamination procedure. Ensure that the Certificate of Decontamination as well as the return authorization number (RGA) are sent with the instrument.

Your local Invitrogen representative will keep you up to date with the progress of service and provide you with any further details you might need, e.g., on maintenance, serviceability, troubleshooting and replacement.

9.4.1 Decontamination

Decontamination should be performed in accordance with normal laboratory procedures. Any decontamination instructions provided with the reagents used should be followed.

A decontamination procedure is only recommendable when infectious substances have been in direct contact with any part(s) of the instrument.

9.4.1.1 Certificate of Decontamination

The decontamination procedure is required prior to shipping the instrument to Invitrogen, e.g., for repair. If the instrument is shipped back to Invitrogen it must be accompanied by a dated and signed Certificate of Decontamination, which must be attached to the outside of the package containing the instrument. See Section 7.3 Decontamination procedure.

Failure to confirm decontamination will incur additional labor charges or at worst the items will be returned for proper cleaning.

Before returning any instrument(s) or item(s), ensure that they are fully decontaminated. Confirm A or B status:

Name: ______________________________

Address: ______________________________

Tel./Fax: ______________________________

Name: ______________ Serial no.: ______________

A)
I confirm that the returned items have not been contaminated by body fluids, toxic, carcinogenic or radioactive materials or any other hazardous materials.
B)
I confirm that the returned items have been decontaminated and can be handled without exposing the personnel to health hazards.

Materials used in the unit: Chemicals + Biological • Radioactive *)

Specific information about contaminants: ______________________________

____

____

Decontamination procedure[1]: ______________________________

____

____

Date and place: ______________________________

Signature: ______________________________

Name (block capitals): ______________________________

* The signature of a Radiation Safety Officer is also required when the unit has been used with radioactive materials.

This unit is certified by the undersigned to be free of radioactive contamination.

Date and place: ______________________________

Signature: ______________________________

Name (block capitals): ______________________________

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[1] Please include decontaminating solution used.

10 WARRANTY CERTIFICATE

10.1 Warranty Certificate

The BeadRetriever is fully guaranteed against defective parts and materials, including defects caused by poor workmanship, for a period of one year from the date of delivery.

Invitrogen will repair or replace defective parts or materials during the term of warranty at no extra charge for materials and labor provided that the products were used and maintained in accordance with Invitrogen Instructions for Use. The warranty is invalid if products have been misused or abused.

For the warranty to be effective, the product must have been purchased either directly from Invitrogen subsidiaries or distributors. The guarantee is not transferable to a third party without prior written approval from Invitrogen.

This guarantee is subject to the following exclusions:

- Any defects caused by normal wear and tear.
- Defects caused by fire, lightning, flood, earthquake, explosion, sabotage, war, riot, or any other occurrence of the type listed above.
- Refurbished products that are subject to different warranty conditions.

THIS WARRANTY IS IN LIEU OF ALL OTHER EXPRESSED OR IMPLIED WARRANTIES, INCLUDING BUT NOT LIMITED TO ANY IMPLIED WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. The seller is not liable for any loss or damage arising out of or in connection with the use of the product or other indirect damages.

These warranty terms and conditions can be obtained from your local Invitrogen representative.

This card acts as a warranty certificate.

10.2 Warranty limitations

- Consumables are not included in the warranty.
- The warranty does not extend beyond the expiration date of the reagent kit.

11 ORDERING INFORMATION

Code	Instrument / System
159.50	BeadRetriever™, 100–240 V

11.1 List of accessories

Code	Item	Quantity
1508270	*BeadRetriever™ User Manual*	1
159.51	BeadRetriever™ Tubes and Tips	1

11.2 List of spare parts

Code	Item	Quantity
159.52	Tube-strip tray = Tube Rack	1

11.3 List of recommended spare parts

Code	Item*	1 – 2 unit(s) / year	10 units / year
1091 910	Tooth belt T2.5/330		1
1092 250	Tooth belt T2.5/265		1
2004 460	PCB PSP-01		1
2004 470	PCB CSU-02	1	2
2004 480	PCB CSU-01		1
2104 0811	Power supply	1	2
2104 0820	H-motor assembly		1
2104 0850	M-motor assembly		1
2407 0740	Free wheel assembly	1	3
2407 2550	Magnetic plate assembly		1

* = Contact your local Invitrogen representative

12 GLOSSARY AND ABBREVIATIONS

AIMS	Automated immunomagnetic separation.
BGA	Brilliant Green Agar.
BPW	Buffered Peptone Water.
BRILA	Brilliant Green Bile Lactose Broth.
BSA	Bismuth Sulfite Agar or Bovine Serum Albumin.
CT	Cefixime-Tellurite; e.g., CT supplement and CT-SMAC.
EMB	Eosin Methylene Blue.
EPEC	Enterotoxin producing *Escherichia coli*.
GN	Gram-negative; e.g., GN broth.
HC	Haemorrhagic Colitis Agar; e.g., mHC = modified Haemorrhagic Colitis Agar.
HE	Hektoen Enteric Agar.
IMS	Immunomagnetic separation.
Magnetic rod	The rods that are magnetic and collect magnetic beads. The rods do not collect the beads on their own; the magnetic rods must always be protected by a tip comb.
PBST (PBS-Tween)	Phosphate Buffered Saline containing 0.05% Tween® 20.
RVS	Rappaport Vassilliadis Soya peptone broth.
SMAC	Sorbitol MacConkey media.
Tip comb	Protects the magnetic rod. A disposable tip comb always has to be fitted into the tip comb holder slot during processing.
TBST	Tris buffered saline with Tween; e.g., TBST-BSA.
TSB	Tryptone Soya Broth; e.g., mTSB = modified Tryptone Soya Broth.
Tube strip	The disposable tubes where all the reagents and samples are located and where the processing takes place.
Tube-strip tray (Tube rack)	A removable tube-strip tray or tube rack that holds 15 tube strips (*Fig. 6.3* and *Fig. 6.5*).
VCC	Vancomycin Cefsulodin Cefixime.
VTEC	Verocytotoxin producing *Escherichia coli*.
XLD	Xylose Lysine Desoxycholate medium.

12.1 Keywords for web pages

AIMS
automated IMS
BeadRetriever
Cryptosporidium
Dynabeads
***E. coli* O157**
EPEC/VTEC
IMMUNOMAGNETIC SEPARATION
Giardia
IMS
Listeria
magnetic beads
magnetic separation
processing
processor
purification
Salmonella
separation
separator
superparamagnetic beads
Thermo Fisher Scientific
Thermo Scientific

12.2 Literature

A collection of articles illustrating the use of Dynabeads-based IMS protocols for the isolation of bacteria and protozoa is available at our website. These articles include protocols for:

- *E. coli O157* from Meat
- *E. coli O157* & other EPEC/VTECs
- *Giardia*
- *Listeria*
- *Salmonella* spp.
- *Salmonella* and Eggs
- *Cryptosporidium*

Visit our website at www.invitrogen.com/microbiology.

13 INDEX

14 TABLE OF FIGURES

15 APPENDIX A. Application Protocols for BeadRetriever using Dynabeads® for selective concentration of target microorganisms

Dynabeads-based products for microbiology have been designed for both manual and automated immunomagnetic separation of specific target organisms from food, feed and environmental samples. Either process can selectively concentrate the specific target organisms from enriched sample matrices. Before proceeding with automated immunomagnetic separation (AIMS), carefully read the operating instructions of the BeadRetriever and the user instructions accompanying each Dynabeads microbiology product. The instrument currently supports only Dynabeads-based products for the selective concentration of *Salmonella*, *Listeria, Legionella* species, *Cryptosporidium*, *Giardia* and the verocytotoxin producing *Escherichia coli* (VTEC) serotypes O157, O145, O111, O103 and O26.

The specially designed disposable tip combs and tube strips that are needed to use the instrument must be purchased separately. These disposable plastic components can be autoclaved before use. It is recommended that the tube strips should be autoclaved before use. The tips are designed for easy insertion into the slots in the instrument in one direction only. The tubes are designed as a strip containing five tubes per test sample for easy insertion into the designated rack and subsequently into the instrument. Both tip combs and tubes are designed for one-time use only and should be disposed of into a biohazardous waste container.

Application Note

Notice that only 500 µl of the enriched samples is to be added to 500 µl of washing buffer (PBST[1]) in each of the two sample tubes. This will result in a twofold dilution of the 1 ml sample for analysis. This is done to achieve a test sample from samples that are fatty, viscous and/or particulate from which bead recovery would otherwise have been difficult[2]. This twofold dilution results in an optimal sample preparation to achieve optimal bead recovery while performing AIMS. Samples that are otherwise not fatty, viscous and/or particulate could be analyzed without any such dilution. In this case, only 1 ml of the (pre)-enriched sample should be added to the sample tubes. This implies that a total sample volume of 2 ml would be analyzed with a resultant increase in the number of target organisms recovered. This approach might be useful when performing tests where the number of target organisms might be limiting due to competition or a prolonged lag phase due to sub-lethal injury.

In order to avoid cross contamination of the prepared tubes, it is recommended that transfer of samples be performed in a designated area at least one meter from the prepared tubes. It is important that filtered pipette tips are used to transfer samples into the test tubes. It is recommended that the tip combs be removed not less than 10 minutes after the assay has been completed to allow for air-drying before removal.

---

[1] Washing buffer (PBS Tween): 0.15M NaCl, 0.01M Sodium-Phosphate buffer, pH 7.4, with 0.05% Tween-20. (Autoclavable at 121°C for 15 minutes, can be stored under refrigeration). Available in powder form from Sigma Aldrich (No. P3563).

[2] A filter homogenizer bag removes particulate material and fatty substances, which are inhibitory to IMS. For certain foods, for example, meat with bones or dry pasta, a blender is preferred prior to using a homogenizer bag to avoid the risk of perforation. After blending, the contents should be transferred into a filter homogenizer bag or wide screw cap bottle.

Automated IMS Using Dynabeads anti-E. coli O157

Dynabeads anti-*E. coli O157* has been designed for use in both manual and automated immunomagnetic separation of *E. coli O157* from food, feed and environmental samples. Either process can selectively concentrate *E. coli O157* from enriched sample matrices.

Before proceeding with automated immunomagnetic separation, carefully read the operating instructions of the BeadRetriever and the *Instructions for Use* accompanying the Dynabeads anti-*E. coli O157* product. Details regarding buffers, media, other components as well as the protocols are provided with the Dynabeads anti-*E. coli O157 Instructions for Use*.

SAMPLE PREPARATION

<u>Food samples</u>

1. Weigh 25 g of food sample and place into a filter homogenizer bag.
2. Add 225 ml of the desired enrichment medium (e.g., BPW, TSB, or BGBB) and homogenize. Incubate for 6−18 h at 37°C or 41.5°C.
3. Mix the pre-enriched sample thoroughly by homogenizing once more.

<u>Human stool, bovine faeces and environmental swab samples</u>

1. For human and animal stool samples, prepare a 10% suspension in physiological saline and transfer 1 ml into 10 ml of a suitable enrichment broth.
2. Human rectal swab and environmental swab samples should be transferred into 10 ml of a suitable enrichment broth.
3. Enrichment broths should be incubated as described above for food samples.

<u>Water samples</u>

1. Filter 1 liter of water according to standard local procedures.
2. Use a flat-ended forceps to remove the filter and transfer directly into a wide-mouthed bottle.
3. Add 90 ml of BPW or TSB to the contents of the bottle and shake vigorously to dislodge bacteria from the surface of the membrane.
4. Incubate for 6−24 h at 37°C or 41.5°C.
5. The use of a filter aid is recommended for samples that are too turbid for membrane filtration.

AIMS OPERATION PROCEDURE

Place one disposable sample tube strip into a BeadRetriever rack for each sample to be processed and using aseptic technique, dispense reagents into each tube. The tab on the tube strip may be used for labeling samples. Users must read the *Instructions for Use* for each individual Dynabeads anti-*E. coli O157* product before use as follows:

1. Resuspend Dynabeads anti-*E. coli O157* until the pellet in the bottom disappears by vortexing.
2. Aseptically add 10 µl of properly mixed Dynabeads anti-*E. coli O157* into the two sample tubes 1 and 2.
3. Aseptically add 500 µl of wash buffer to sample tubes 1 and 2.
4. Aseptically add 1 ml of wash buffer to tubes 3 and 4 within the strip.
5. Aseptically add 100 µl of wash buffer to the 5th tube.
6. Remove the desired tube from the sample rack and place in a second sample rack (one meter away). Add 500 µl of a test sample to tubes 1 and 2 and transfer the inoculated tube to the first sample rack. Repeat for the remaining samples.
7. Aseptically insert the sterile protective tip combs into the instrument.
8. Insert the rack with filled tubes into the instrument to lock it in place.
9. Check that everything is properly aligned and close the instrument door.
10. Select the **EPEC/VTEC program** sequence by scrolling with the arrow key and press the **START** button.

11. While the instrument is in operation, the door must be kept closed. Each processing step and the total time remaining can be followed on the LC display.
12. At the end of the program run, remove the tube's rack from the instrument and plate one half of the bead-bacteria complexes from the 5th tube onto each of the appropriate plating media as recommended below.
13. Remove the tip combs and discard into a biohazard waste container together with the tube strips.

ISOLATION PROCEDURE

After IMS, transfer the resuspended beads onto each internationally accepted *E. coli O157* culture media plates. It is recommended that two different culture media are used to increase the chances of detecting suspect colonies that have distinct differential features on each media. Invitrogen recommends Sorbitol MacConkey (SMAC) media supplemented with CT-supplement and CHROMagar® O157.

1. Spread the bead-bacteria complexes over one half of the plate with a sterile swab. This ensures the break-up of the bead-bacteria complexes.
2. Dilute further by streaking with a loop. Always carry the loop back into the previously streaked quadrant several times to ensure that the beads reach a fresh unstreaked quadrant.
3. Incubate the plates at 35−37°C for 18−24 h. Read the plates for suspect *E. coli O157* colourless colonies on CT-SMAC and pink-mauve coloured colonies on CHROMagar O157.

The choice of plating media has been based on some distinct characteristics of *E. coli* 157:H7. It is the only *E. coli* among clinical isolates which does not ferment sorbitol within 24 h and which is glucuronidase negative. The organisms are resistant to potassium tellurite and cefixime.

Presumptive *E. coli O157* colonies must be confirmed by standard biochemical and serological testing.

Automated IMS Using Dynabeads EPEC/VTEC Product Series (O145, O111, O103 and O26)

The Dynabeads EPEC/VTEC Product Series is specifically developed for rapid selective concentration of non-O157 *Escherichia coli* serotypes O145, O111, O103 and O26 using the BeadRetriever from food, feed and environmental samples. The products may also be used to perform manual IMS. Either process can selectively concentrate the targeted non-O157 *E. coli* serotypes from 24 h pre-enriched sample matrices at 42°C.

Before proceeding with automated immunomagnetic separation, carefully read the operating instructions of the BeadRetriever and the *Instructions for Use* accompanying each Dynabeads EPEC/VTEC product. Details regarding buffers, media, other components as well as the protocols are provided with the Dynabeads EPEC/VTEC product *Instructions for Use*.

SAMPLE PREPARATION

Food samples

1. Weigh 25 g of food sample and place into a filter homogenizer bag.
2. Add 225 ml of Buffered Peptone Water (BPW).
3. Incubate at 42°C for 24 h.
4. Mix the pre-enriched sample thoroughly by homogenizing once more.

Human stool, bovine faeces and environmental swab samples

Whole stool specimens must be refrigerated as soon as possible after collection and must be examined within 1–2 h of collection. If they cannot be examined within 1–2 h, whole stool samples or a swab of the stool or rectal swabs should be placed in a transport medium (e.g., Stuart's, Cary Blair, etc.) and held refrigerated until examination within 2–3 days (d). If samples are held longer than 3 days before examination, they should be frozen at -70°C. Specimens in transport medium should not be left at ambient temperature.

1. Transfer 1 ml of human liquid stool sample into 10 ml of BPW.
2. For solid human stool samples and bovine faeces, prepare a 10% suspension in physiological saline and transfer 1 ml into 10 ml BPW.
3. Human rectal and environmental swab samples should be transferred into 10 ml of BPW.
4. Human stool, bovine faeces and environmental samples must be pre-enriched for 24 h at 42°C.

Water samples

1. Filter 1 liter of water according to local standard procedures.
2. Use a flat-ended forceps to remove the filter and transfer directly into a wide-mouthed bottle.
3. Add 90 ml of BPW to the contents of the bottle and shake vigorously to dislodge bacteria from the surface of the membrane.
4. Incubate at 42°C for 24 h.
5. The use of filter aid is recommended for samples that are too turbid for membrane filtration.

AIMS OPERATION PROCEDURE

Users must aseptically dispense all reagents and samples into the tubes sequentially after the tube strips are fitted into the rack. Users must read the *Instructions for Use* for each individual Dynabeads EPEC/VTEC products before use as follows:

1. Aseptically add 10 µl of properly mixed specific Dynabeads EPEC/VTEC product into the two sample tubes 1 and 2.
2. Aseptically add 500 µl of washing buffer (PBST) to sample tubes 1 and 2.
3. Aseptically add 1 ml of washing buffer to tubes 3 and 4 within the strip.
4. Aseptically add 100 µl of washing buffer to the 5th tube.
5. Remove the desired tube from the sample rack and place in a second sample rack (one meter away). Add 500 µl of a test sample to tubes 1 and 2 and transfer the inoculated tube to the first sample rack. Repeat for the remaining samples.
6. Aseptically insert the sterile protective tip combs into the instrument.
7. Insert the rack with filled tubes into the instrument to lock it in place.
8. Check that everything is properly aligned and close the instrument door.
9. Select the **EPEC/VTEC program** sequence by scrolling with the arrow key and press the **START** button.
10. While the instrument is in operation, the door must be kept closed. Each processing step and the total time remaining can be followed on the LC display.
11. At the end of the program run, remove the tube rack (i.e., tube-strip tray) from the instrument and plate one half of the bead-bacteria complexes from the 5th tube onto each of the appropriate plating media as recommended below.
12. Remove the tip combs and discard into a biohazardous waste container together with the tube strips.

ISOLATION OF EPEC/VTEC OTHER THAN O157 VTEC

After automated IMS, transfer all the resuspended bead-bacteria complexes onto blood agar or one half of the bead-bacteria complexes onto blood agar and the remaining half onto anyone of the following plating media: CHROMagar O157 (Invitrogen); MacConkey agar; modified Haemorrhagic Colitis agar (mHC); Eosin Methylene Blue (EMB), and Chromocult Coliform Agar (Merck).

1. Spread the bead-bacteria complexes over one half of the plate with a sterile swab. This is to ensure the break-up of the bead-bacteria complexes. Dilute further by streaking with a loop. Always carry the loop back into the previously streaked quadrant several times to ensure that the beads are streaked into a fresh unstreaked quadrant. See *Fig. 15.1*.
2. Incubate the plates at 35–37°C for 18–24 h.
3. Proceed to the next section.

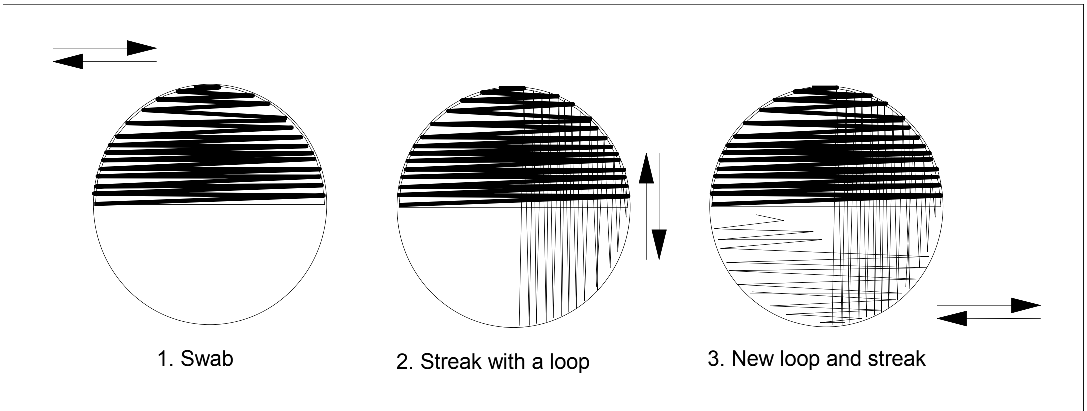


Fig. 15.1 Swab-streak technique

PRESUMPTIVE IDENTIFICATION AND CONFIRMATION OF NON-O157 VTEC

1. Add 10 µl of physiological saline onto a glass slide placed on a dark background. Two or three tests may be performed on one slide.
2. Transfer a sweep of mixed growth from the first half of the blood agar plate or a single colony onto the slide and make a smooth milky suspension.
3. Observe for autoagglutination.
4. In the absence of any autoagglutination, add 10 µl of the specific OK EPEC/VTEC antiserum provided in the Colony Verification kit to the suspension and mix well. Observe for agglutination by tilting the slide for 1–30 seconds.
5. If autoagglutination occurs, test one to five distinct individual colonies (see step 7 below).
6. A visible agglutination reaction within 30 seconds is a strong indication of a presumptive positive sample.
7. When testing a sweep of mixed growth, confirm the initial presumptive result by testing one to five distinct individual colonies from the blood agar plate in a similar manner using the specific OK antiserum. If no distinct colonies could be picked, plate further for purity from the other plating media onto blood agar and proceed as described above.
8. The reaction is read with the naked eye by holding the slide in front of a light source against a black background (i.e., indirect illumination).
9. A **positive** reaction is seen as a visible agglutination. A **negative** reaction is persistence of the homogeneous milky turbidity. A late or weak agglutination should be considered negative.
10. Plate the agglutination-positive colonies further for purity and confirm them by standard biochemical, serological and DNA tests (e.g., PCR).

Automated IMS Using Dynabeads anti-Salmonella

Dynabeads anti-*Salmonella* has been designed for use in both manual and automated immunomagnetic separation of *Salmonella* from food, feed and environmental samples. Either process can selectively concentrate *Salmonella* from enriched sample matrices.

Before proceeding with automated immunomagnetic separation, carefully read the operating instructions of the BeadRetriever and the *Instructions for Use* accompanying the Dynabeads anti-*Salmonella* product. Details regarding buffers, media, other components as well as the protocols are provided with the Dynabeads anti-Salmonella *Instructions for Use*.

SAMPLE PREPARATION

<u>Food, feed and environmental samples</u>

1. Weigh 25 g of sample material and place into a stomacher bag with filter and add 225 ml of pre-enrichment broth. (Invitrogen recommends buffered peptone water as a pre-enrichment broth).
2. Mix well using the stomacher apparatus (a stomacher bag with filter removes particulate matter as well as fatty components and allows easy pipetting of clear aliquot for analysis). For certain foods, for example bony meat, pasta, etc. a blender is preferred prior to using a stomacher bag to avoid the risk of perforation. However, after blending the contents should be transferred into a stomacher bag with a filter.
3. For environmental samples using a swab, place the swab into 10−50 ml of pre-enrichment broth and incubate as described below.
4. Incubate the prepared sample in the stomacher bag for 18−24 hours at 37°C.
5. Mix the pre-enriched sample thoroughly, by homogenising once more.
6. Pipette 1 ml aliquot of the filtered suspension for the immunomagnetic separation procedure.
7. Change to a new pipette for each new sample.

<u>Method for shell eggs</u>

1. Wash dirty eggs with a stiff brush under running water, and dry with a paper towel.
2. Dip the eggs into 70% ethanol for 5−10 seconds and allow to dry. Alternatively follow any standard procedure for disinfecting shell eggs.
3. Aseptically crack open the eggs and mix/ blend thoroughly both white and yolk.
4. Add Ferrous Sulphate ($FeSO4$) solution to a final concentration of 35 mg/L.
5. Pre-incubate the egg mixture at 37°C for 6 hours.
6. After pre-incubation, mix the egg mixture thoroughly.
7. Dilute an aliquot five fold with wash buffer or buffered peptone water and use 1 ml of this dilution for IMS analysis.
8. Use a new pipette or a new pipette tip for each sample to avoid cross-contamination.
9. Re-incubate the remaining undiluted egg mixture overnight at 37°C.

AIMS OPERATION PROCEDURE

Users must read the *Instructions for Use* for each individual Dynabeads anti-Salmonella product before use as follows:

1. Load one BeadRetriever sample tube strip for each sample into a sample rack.
2. Resuspend Dynabeads anti-*Salmonella* by vortexing until the pellet in the bottom of the tube disappears and aseptically add 10 µl of properly mixed Dynabeads anti-*Salmonella* into sample tubes 1 and 2.

Note: For the *Shell Eggs* method, add **20 µl** of properly mixed Dynabeads anti-*Salmonella* into sample tubes 1 and 2.

3. Aseptically add 500 µl of wash buffer to sample tubes 1 and 2.
4. Aseptically add 1 ml of wash buffer to tubes 3 and 4 within the strip.
5. Aseptically add 100 µl of wash buffer to tube 5.
6. For each sample remove the labelled sample tube strip from the sample rack and place in a second sample rack (one meter away). Add 500 µl of the test sample to tubes 1 and 2 and return the inoculated tube to the first sample rack. Repeat for the remaining samples.
7. Aseptically insert the sterile protective sample tip combs into the instrument.
8. Insert the rack with filled tubes into the instrument to lock it in place.
9. Check that everything is properly aligned and close the instrument door.
10. Select the **SALMONELLA program** sequence by scrolling with the arrow key and press the **START** button.

Note: For the *Shell Eggs* method select the Salmonella (eggs) program from the BeadRetriever menu.

11. While the instrument is in operation, the door must be kept closed. Each processing step and the total time remaining can be followed on the LC display.
12. At the end of the program run, remove the sample rack from the instrument.
13. For each sample process the bead-bacteria complexes either using the RAPID or ENHANCED methods described below.
14. Remove the sample tip combs and discard into a biohazard waste container together with the tube strips.

ISOLATION PROCEDURE

The ENHANCED Method

This is the recommended method for all food and environmental samples. Presumptive *Salmonella* positive results are available three days after receipt of samples.

1. Transfer the concentrated, resuspended bead-bacteria complexes into 10 ml of Rappaport Vassilliadis Soya peptone broth (RVS) and incubate at 42°C for 18−24 hours.
2. Follow standard procedure for isolation by spreading a loopful of RVS culture onto any *Salmonella* plating media, such as Brilliant Green agar (BGA), Xylose-Lysine-Deoxycholate agar (XLD), Bismuth Sulphite agar (BSA), Hektoen agar (HE), etc.

The RAPID Method

This is recommended for processed foods known to harbour none or low levels of background flora only. Presumptive *Salmonella* positive results are available two days after receipt of samples.

1. Transfer 50 µl of the resuspended bead-bacteria complex onto each of two *Salmonella* selective agar plates (BGA, XLD, BSA, HE, etc.).
2. Interpretation of presumptive results depends on the skill of the user to correctly differentiate the isolated colonies based on typical *Salmonella* morphology. Suspect colonies must be confirmed by standard biochemical and serological test methods.

Automated IMS Using Dynabeads anti-Listeria

Dynabeads anti-*Listeria* has been designed for use in both manual and automated immunomagnetic separation of *Listeria* species from food, feed and environmental samples. Either process can selectively concentrate *Listeria* from enriched sample matrices.

Before proceeding with automated immunomagnetic separation, carefully read the operating instructions of the BeadRetriever and the *Instructions for Use* accompanying the Dynabeads anti-*Listeria* product. Details regarding buffers, media, other components as well as the protocols are provided with the Dynabeads anti-*Listeria Instructions for Use*.

SAMPLE PREPARATION

Food samples

1. Weigh 25 g of food sample and place into a filter homogenizer bag.
2. Add 225 ml of Half-Fraser broth and homogenize.
3. Incubate the prepared sample at 30°C for 24 h.
4. Mix pre-enriched samples thoroughly by homogenizing once more.

Environmental samples

1. Take a swab sample from any surface material or filter 10 liters of water through a membrane filter.
2. Place the swab or filter into an appropriate container filled with 10–50 ml of Half-Fraser broth. Incubate for 24 h at 30°C.
3. Mix by shaking vigorously.

AIMS OPERATION PROCEDURE

Users must aseptically dispense all reagents and samples into the tubes sequentially after the tube strips are fitted into the rack. Users must read the *Instructions for Use* for Dynabeads anti-*Listeria* before use as follows:

1. Aseptically add 10 µl of properly mixed Dynabeads anti-*Listeria* into the two sample tubes 1 and 2.
2. Aseptically add 500 µl of washing buffer (PBST) to sample tubes 1 and 2.
3. Aseptically add 1 ml of washing buffer to tube 3 within the strip.
4. Aseptically add 100 µl of washing buffer to the 5th tube.
5. For each sample remove the labelled sample tube strip from the sample rack and place in a second sample rack (one meter away). Add 500 µl of a test sample to tubes 1 and 2 and transfer the inoculated tube to the first sample rack. Repeat for the remaining samples.
6. Aseptically insert the sterile protective tip combs into the instrument.
7. Insert the rack with filled tubes into the instrument to lock it in place.
8. Check that everything is properly aligned and close the instrument door.
9. Select the **LISTERIA program** sequence by scrolling with the arrow key and press the **START** button.
10. While the instrument is in operation, the door must be kept closed. Each processing step and the total time remaining can be followed on the LC display.
11. At the end of the program run, remove the tube rack (i.e., tube-strip tray) from the instrument and plate one half of the bead-bacteria complexes from the 5th tube onto each of the appropriate plating media as recommended below.
12. Remove the tip combs and discard into a biohazardous waste container together with the tube strips.

ISOLATION PROCEDURE

The resuspended Dynabeads-bacteria complexes are now ready for plating. Transfer 50 µl of the resuspended bead-bacteria complexes from the 5th tube onto two *Listeria* plating media, one of which should be Palcam agar and plate by standard streaking with a loop or the swab-streak technique (*Fig. 15.1*). All inoculated plating media must be incubated at 37°C. The plates are read after 24 h and if necessary after 48 h for presumptive *Listeria* colonies. Total analysis time from sample receipt to presumptive results is 48 h.

CONFIRMATION

The presumptive *Listeria* colonies must be confirmed by standard biochemical and serological testing or by genetic fingerprinting to identify the species.

Automated IMS Using Dynabeads anti-Legionella

Dynabeads anti-*Legionella* has been designed for use in both manual and automated immunomagnetic separation of *Legionella* species (spp.) from water samples and water sample concentrates.

Before proceeding with automated immunomagnetic separation, carefully read the operating instructions of the BeadRetriever and the Instructions for Use accompanying the Dynabeads anti-*Legionella* product. Details regarding buffers, media, other components as well as the protocols are provided with the Dynabeads anti-*Legionella Instructions for Use*.

SAMPLE PREPARATION

This test protocol has been designed for use with 1 ml of water sample concentrates, spiked water samples, environmental swab samples and dilute suspensions of target, non-target or reference organisms. This protocol is also suitable for samples which have been acid or heat-treated. If the sample has been stored below room temperature, leave sufficient time to allow the sample to equilibrate to room temperature.

Samples may be prepared by standard filtration and/or centrifugation methods appropriate to meet local regulations.* This is recommended where target cell numbers are expected to be below 1000 colony forming units (cfu) per liter.

Environmental swab samples may be eluted into a suitable volume of Maximum Recovery Diluent prior to IMS. **Note:** Maximum Recovery Diluent is an isotonic diluent containing a low level of peptone used for maintaining the viability of organisms during dilution procedures. Please use Oxoid Cat. No. CM733, Difco Cat. No. 218971 Dehydrated – 500 g, or equivalent.

*BS ISO 11731-2:2004, BS 6068-4.18:2004 Water quality. Detection and enumeration of *Legionella*. Direct membrane filtration method for waters with low bacterial counts.

BS 6068-4.12:1998, ISO 11731:1998 Water quality. Microbiological methods. Detection and enumeration of *Legionella* (Current, Work In Hand) http://bsonline.techindex.co.uk.

AIMS OPERATION PROCEDURE

Place one disposable sample tube strip into a BeadRetriever rack for each sample to be processed and using aseptic technique, dispense reagents into each tube. The tab on the tube strip may be used for labeling samples. Users must read the *Instructions for Use* for Dynabeads anti-*Legionella* before use as follows:

1. Add 100 µl of Sample buffer to sample tube 1.
2. Vortex Dynabeads anti-*Legionella* for a minimum of 10 seconds to mix.
3. Add 50 µl of Dynabeads anti-*Legionella* into sample tube 1.
4. Add 1 ml of Wash buffer to tubes 2 and 3 within the strip.
5. Add 100 µl of Wash buffer to tube 4.
6. Add 1 ml of sample aliquot to tube 1 (to avoid cross contamination between samples, it is recommended that sample transfer is performed at least 1 meter from the other prepared tube strips).
7. Insert the sterile disposable protective tip combs into the instrument. Each comb should click into position.
8. Insert the rack with filled tubes into the correct position in the instrument.
9. Check that everything is properly aligned and close the instrument door.
10. Select the **ENVIRONMENTAL program** sequence by scrolling with the arrow key and press the **START** button.
11. While the instrument is in operation, the door must be kept closed. Each processing step and the total time remaining can be followed on the LC display.
12. At the end of the program run, remove the tube rack from the instrument.

13. Remove the tip combs and tube strips and discard into an appropriate biohazardous waste container and autoclave prior to disposal.

Safety Note: During operation the BeadRetriever does not create a biohazardous aerosol and may be operated safely on the laboratory bench. For further information see: http://www.hpa.org.uk/srmd/div_esl_su/pdf_guidance2.htm.

ISOLATION PROCEDURE

Plate the bead-bacteria complexes from tube 4 onto an appropriate *Legionella* selective media (for example, GVPC agar) using a standard plating technique and according to local procedures. Culture plates should then be incubated according to standard procedures.

CONFIRMATION

Interpretation of presumptive results depends on the skill of the user to correctly differentiate the isolated colonies based on the typical *Legionella* morphology. Suspect colonies must be confirmed by standard biochemical and serological test methods.

Automated IMS using Dynabeads Anti-Giardia and Anti-Cryptosporidium

Dynabeads anti-*Giardia* and anti-*Cryptosporidium* have been designed to be used for the isolation and detection of *Giardia* cysts and *Cryptosporidium* oocysts from environmental water samples. These immunomagnetic beads can be used to simultaneously isolate both *Giardia* and *Cryptosporidium* or to isolate *Cryptosporidium* only. These beads can be used for both manual and automated protocols.

Before proceeding with automated immunomagnetic separation, carefully read the operating instructions of the BeadRetriever and the *Instructions for Use* accompanying the Dynabeads anti-*Giardia* and Dynabeads anti-*Cryptosporidium* (GC Combo, Cat. No. 730-02, 730-12) or the Dynabeads anti-*Cryptosporidium* (Cat. No. 730-01, 730-11) products. Details regarding buffers, media, other components as well as the protocols are provided with the Dynabeads anti-*Giardia* and anti-*Cryptosporidium Instructions for Use*.

SAMPLE PREPARATION

This test has been designed for use with water samples produced and eluted via the standard method stated in the EPA method 1622/1623 and UK DETR Water Supply Regulation 2000 SI No. 3184 (England) and 2001 SI No. 3911 (Wales).

For labs using the IDEXX Filta-Max® filtration system
The protocols followed using the IDEXX Filta-Max filtration system results in a 50 ml concentrate, which is then centrifuged according to standard procedure. After centrifugation the supernatant is removed via gentle aspiration to 3 ml volume. The pellet size is recorded.

For labs using the Pall Envirochek® or Envirochek HV filter capsule
For protocols followed using the Pall Envirochek or Envirochek HV filter capsule, filter and elute the sample as usual. After centrifugation, re-suspend the pellet in 30 ml of buffer and transfer to a 50 ml conical tube. Perform two additional rinses (10 ml each) of the centrifuge tube to recover all remaining sediment. Centrifuge the sample again at 1500 x G. Allow the rotator to coast to a stop – do not use the brake. Re-record the pellet volume on the bench sheet. Proceed as described above for the Filta-Max filter.

If the pellet is above 0.25 ml, then split the sample into sub-samples, respectively; for example, if the pellet is 0.5 ml, split into two sub-samples of 0.25 ml pellet each.

The 3 ml concentrate is then vortexed thoroughly to re-suspend the pellet.

AIMS OPERATION PROCEDURE

Place one disposable sample tube strip into a BeadRetriever rack for each sample to be processed. The tab on the tube strip may be used for sample labeling. Users must read the *Instructions for Use* for Dynabeads anti-*Giardia* and anti-*Cryptosporidium* before use as follows:

1. To tubes 1−3 add 100 µl of 10X SL® Buffer A and 100 µl of 10X SL Buffer B.
2. To tube 4 add 1 ml of 1X SL Buffer A.
3. To tube 5 add 100 µl of 0.1 N HCL.
4. Split the 3 ml sample concentrate across tubes 1−3; 1 ml into each tube 1−3.
5. Add up to 300 µl RO water to the sample concentrate tube. Vortex the concentrate tube to re-suspend and wash any debris left behind. This produces the rinsate.
6. Split the rinsate across tubes 1−3; up to 100 µl to each tube 1−3.
7. Add 33 µl of Dynabeads anti-*Cryptosporidium* to each tube 1−3. For *Cryptosporidium* only detection, proceed to step 9.
8. For dual *Giardia* and *Cryptosporidium* detection within the same assay, also add 33 µl of Dynabeads anti-*Giardia* to each tube 1−3.
9. Insert the protective tip combs into the BeadRetriever.
10. Insert the rack containing the tube strips into the BeadRetriever.
11. Check that everything is properly aligned and close the instrument door.

12. Select the ***GC Combo*** **program** by scrolling with the arrow keys and then press the **START** button.
13. During instrument operation the door should be kept closed at all times.
14. The display will show the time left for each stage of the automated IMS protocol. The stages of the *GC Combo* protocol in order are: Mixing stage, Bead isolation, Wash stage and then dissociation stage.
15. After the dissociation stage the machine will beep once finished.
16. At the end of the program run, open the instrument door and carefully remove the rack containing the sample tube strips.

POST AUTOMATED IMS PROCESS

1. Remove each sample tube strip one at a time and carefully remove the 100 µl 0.1 N HCL from tube 5.
2. Spot this 100 µl onto Dynal® Spot-On (Cat. No. 740.04) slides containing 10 µl 1 N NaOH.
3. Repeat this for all samples processed onto separate slides per sample.
4. Then incubate the slides according to standard procedure; 30 min at 41°C.
5. Once dry, then fix the slides according to standard procedure; 25 µl methanol.
6. Once the slides are fixed, stain with FITC monoclonal antibody for *Giardia* and *Cryptosporidium*. Follow the manufacturer's instructions for FITC staining.
7. After staining with FITC, then stain the slides with 4',6-diamidino-2-phenylindole (DAPI) according to standard procedure; 50 µl for 2 minutes.
8. Wash the slides and mount with cover slip according to standard procedure.
9. The slides are then ready for oocysts enumeration via Fluorescence assay (FA) microscopy and Normanski differential interference contrast (DIC) microscopy.

ADDITIONAL CONFIRMATION PROCEDURE

After samples are enumerated on the slides, you can further process the sample to get information on the species or genotype of the *Cryptosporidium* oocysts captured by AIMS. Using our *Cryptosporidium* genotyping kit (Cat. No. 740-05 Complete Kit, Cat. No. 740-06 Starter Kit, Cat. No. 740-07 Reagents) the user can isolate DNA from the oocysts adhered to the slide. This material is then amplified with unique PCR primers to amplify a *Cryptosporidium* specific target. This target is then digested with three distinct restriction enzymes to produce bands of different sizes. These bands are resolved by gel electrophoresis. Based on the banding pattern, the user can achieve a presumptive species or genotype ID for the organisms present on the slide. Refer to the *Cryptosporidium* Genotyping kit manual for further information.

16 APPENDIX B. BRIEF USER'S GUIDE

- Switch the BeadRetriever on (p. 17).
- Pipette the samples, reagents and washing buffers into the tube strips (p. 19, 40).
- Load the tube-strip tray(s) onto the instrument (p. 19).
- Insert the tip comb(s) into the tip comb holder slot(s) (p. 19).
- Select the desired protocol from the keypad and display (p. 18).
- Press the **START** button (p. 19).
- After the end of the run, remove the tube strips and tip combs (p. 21).
- Maintain your BeadRetriever instrument regularly (p. 22).



Fig. 16.1 BeadRetriever keypad

17 APPENDIX C. BEADRETRIEVER FEEDBACK FORM

Cat. no.	Serial no.
PURCHASED BY	**PURCHASED FROM**
Company/Institute	Distributor
Department	
Address	Address
Tel.	Tel.
Fax	Date of delivery

Internet home page

Date of purchase

Your research area

Dr. ☐ Mr. ☐ Mrs. ☐ Ms. ☐ Job title/Position

Surname (block capitals) | First name (block capitals)

Internet e-mail address

	Excellent	Above expectations	As expected	Below expectations	Comments
Reagent kit/Instructions	☐	☐	☐	☐	
Instrument/User manual	☐	☐	☐	☐	
Operational reliability	☐	☐	☐	☐	
Design	☐	☐	☐	☐	
Ease of use	☐	☐	☐	☐	
Operational costs	☐	☐	☐	☐	
Customer support	☐	☐	☐	☐	

Further instrument/system developments desired:

Further applications desired:

Where did you first learn about the product?

Would you like to receive information about other Invitrogen products?

18 APPENDIX D. ADDRESSES

Invitrogen on the Internet

For the latest information on the BeadRetriever as well as Dynabeads products and services, visit our worldwide web site on the Internet at:

http://www.invitrogen.com/microbiology

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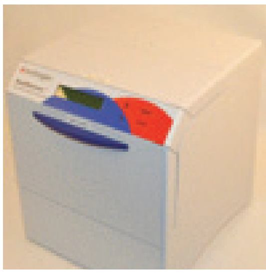
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